

Flow Cytometry Core, MRC 1295
Moffitt Cancer Center
12902 Magnolia Drive
Tampa, FL 33612

TITLE: Standard operating procedure (SOP) for the start-up, maintenance, acquisition, and shutdown of the Amnis ImageStreamX Mark II.

EFFECTIVE DATE: 21st August, 2024

I. PURPOSE

This SOP details the procedure for the start-up, quality control, maintenance, acquisition, and shutdown of the Amnis ImageStreamX Mark II (Cytek Biosciences) imaging flow cytometer.

II. REFERENCES

1. <https://cytekbio.com/pages/imagestream>
2. <https://www.youtube.com/watch?v=otFE2CvWBaw>
3. <https://pubmed.ncbi.nlm.nih.gov/18165167/>
4. <https://www.nature.com/articles/s43586-022-00167-x>

III. MATERIALS AND REAGENTS

PBS: Phosphate Buffered Saline. DiH₂O: Deionized water.

- 1X PBS.
- 10% bleach.
- DiH₂O.
- Coulter Clenz®.
- 70% isopropanol.
- Speed Bead ImageStreamX system calibration reagent. (**Cat#CN-0440-01**).
- 1.5ml siliconized Tubes

Reagent	Name	Source*	Catalog #
Cleanser	Coulter Clenz®	Beckman Coulter	8546929
Debubbler	70% Isopropanol	VWR	42101
Sterilizer	0.4-0.7% Hypo-chlorite	VWR	JT9416-1
Sheath	PBS	Invitrogen	14190
Rinse	deionized water		

IV. PROCEDURE

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A. START-UP

1. Open the front panel of the instrument and check that the rinse bottle is full of DiH₂O, the sheath bottle is full of 1X PBS, the sterilizer bottle is full of 10% bleach, the cleanser bottle is full of Coulter Clenz, the debubbler bottle is full of 70% isopropanol, and Speed Beads are loaded, and the waste bottle (with 160 ml of bleach) is empty.



2. Press the green power button inside the front door to turn on the instrument.
3. Turn on the computer and Log in using the password.
4. Double click the ISX icon (INSPIRE software) to open the acquisition software.

B. QUALITY CONTROL

1. Once the instrument power is ON, computers are connected and ISX Icon (INSPIRE software) is open, click "**STARTUP**" tab in the lower right corner of the software to initialize the instrument and fluidics startup.
2. Once the startup is initialized and calibration tests completes and it passes then green dot appears next to **ASSIST**, if it fails then that dot appear as 'RED' or if some of the channels fail it can show as "Orange."

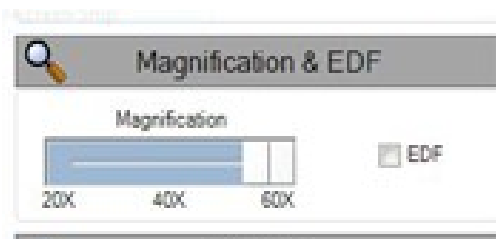
ASSIST

4. If ASSIST fails on some of the parameters, then those parameters need to be re-run individually by double clicking on them.
5. If it fails several times, follow the start-up procedure all over again. If the problem persists, then call Cyttek instrument service.
6. For additional help with calibration, please see the Troubleshooting section (**Ch-5, Page 77**) provided in INSPIRE® software manual.

C. DATA ACQUISITION

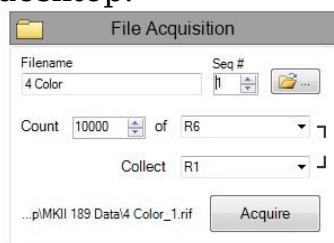
1. Open an existing template (if any) in the software by going to File > Open Template. A template can be created from scratch or modified using an existing template.
2. If FCS files are also required, then check "Generate FCS file" under the file tab along with RIF.
2. Click on the Objective lens needed for the study under "Magnification" (20x, 40x, 60x).

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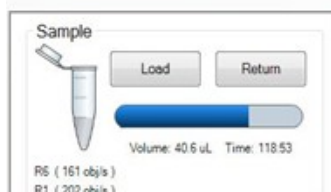
Wait till the instrument reinitializes with new settings.

3. Under the File Acquisition tab, Select “Browse” and make folder on the desktop.



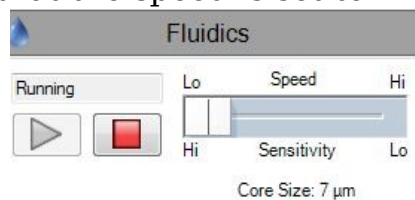
Enter the name of sample and number of cells to be acquired. User can change the storage gate by clicking selecting on collect gate.

4. Click “Load” on the top of the software.



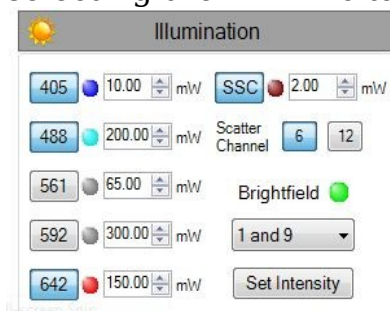
The loader will be released, then load the sample tube (1.5 ml tube) with all the fluorochromes and with the brightest fluorescence.

5. Ensure that the speed is set to “Low” during experiment set-up under



“Fluidics”.

6. Turn on each laser being used under “Illumination” for the experiment by selecting them in Excitation Lasers on the right.



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7. Activate the channels required for the instrument by clicking on it (turns light blue when On or grey when Off) and start with the default **highest** power settings (check the default power settings by bringing the cursor on dots next to respective lasers). Always include Ch01 and Ch09 (brightfield channels) to have spatial coordination between the two cameras.

Note: By default, Brightfield is detected in channels 1 and 9, but it can be changed to channels 2

and 8, channels 4 and 10. Side scatter channels are on 6 or 12 or can be turned off (SSC laser off) if used as fluorescent channels.

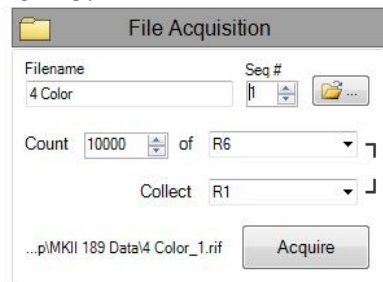
8. Create the histogram plot with "Gradient RMS" on Ch01 parameter and make the gate on "focused cells" (Mostly higher than 40).

9. Create a bivariate plot for Area (X-axis) and Aspect Ratio (Y-axis) on Ch01. Make a gate of on cells showing Aspect Ratio closer to 1.0 (**single cells**) and with intermediate Area (100-600).

10. Make bivariate plots for "**Raw Max Pixel**" for all fluorescent channels.

11. Adjust laser powers if needed to ensure Raw Max Pixel is less than **4e3** so signals are not saturated.

12. Under the Setup tab, enter the number of cells to acquire and the file name.



13. Click "Acquire" to begin saving the events.

14. When acquisition has finished click "Return". Remove the first sample tube and click "OK". Load another tube by clicking "Load", then placing a new tube when the software requests it.

15. Repeat the previous two steps for every samples you have.

Note: If "Load" is selected instead of "Return", the remaining sample will be flushed to waste.

16. Once all the samples have been acquired, run 10% Bleach for one minute followed by running PBS for one minute.

17. Run single color compensation controls. Single color controls can be run manually by turning off Brightfield, SSC laser off and enabling all the channels.

Note: If nuclear dye is in the panel, always run that control as a last tube.

18. Area versus Aspect ratio plots on respective fluorescent channels (gating on single cells) can be used to record single-color controls.

19. After all samples and controls have been acquired, clean the instrument by loading a

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tube with 10% bleach, running for one minute, then loading a sample of diH₂O and running for one minute.

D. SHUTDOWN

1. Ensure that the rinse bottle is full of DiH₂O, the sterilizer bottle is full of 10% bleach, the cleanser bottle is full of Coulter Clenz, and debubbler bottle is full of 70% isopropanol.

2. Empty the waste bottle and refill it with 160 ml of bleach.

3. Ensure that there are no tubes in the uptake port.

4. Click "Sterilize". This procedure is an automated shutdown and should take approximately 45 minutes to complete.

Note: This is adequate for daily shutdown, and the next step is not necessary. If the instrument

will be unused for more than two weeks, continue with the next step.

4. If the instrument will be unused for more than two weeks, replace the bead vial with a vial of

DiH₂O and click "Initialize". Then from the main toolbar select File > Exit and shutdown instrument.

E. TROUBLESHOOTING

1. If there are connectivity issues, turn off the instrument and computers, wait for a few minutes, and then restart them.

2. If no events are detected when running a sample (including Speed Beads), the sample line might be clogged, or the flow cell might contain a bubble.

3. Wash with 10% bleach then with DiH₂O. If the problem persists, go to Maintenance > Debubble to dislodge the clog or bubble.

4. For other troubleshooting issues, follow INSPIRE® software manual **Pages 71-85**.

5. If any other major problem occurs, call Cytex instrument service.

<https://cytekbio.com/pages/contact-list>

V. REVISION HISTORY

21st August, 2024